THE DISTRIBUTION OF B. COLI AND B. AEROGENES TYPES IN POLLUTED AND UNPOLLUTED WATER

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In spite of the unique sanitary importance of the colon bacillus and its allies, the classification of this group has, until very recent years, been in an exceedingly unsatisfactory state. It will be remembered, for example, that MacConkey,[1] and Bergey and Deehan,[2] chiefly on the basis of fermentative characters, subdivided the colon family into over a hundred types. The objections that may be raised against such complex and artificial subdivisions are self-evident. MacConkey in the same paper arranged his types of the colon family in four primary groups on the basis of sucrose and dulcite fermentations. Houston divided the colon family into "typical" and "atypical" varieties depending mainly on the fermentation of sucrose. All of these bases for differentiation are purely arbitrary, and they fail to divide the family into naturally occurring species or groups characteristic of any particular habitat.

Far more fundamental has been the work of Rogers and his associates on the gas production and gas ratios of various members of the colon family. By exact methods of gas analysis, they have demonstrated that, in the fermentation of glucose, colon organisms may on the one hand produce gas which is composed of about equal parts of carbon dioxid and hydrogen, or on the other hand may form a mixture made up of two parts of the former to one of the latter component. Later, Clark and Lubs[3] correlated these different gas ratios with corresponding differences in the final hydrogen ion concentration of the glucose broth medium; thus giving a comparatively simple and practical test for differentiating these types. Following on this development came the work of Levine,[4] of Winslow and Kligler[5] and others

Received for publication Jan. 28, 1918.

[1] Jour. Hyg., 1905, 5, p. 333. MacConkey, A.: Jour. Hyg., 1909, 9, p. 86; Papasotirin, J.: Arch. f. Hyg., 1901, 41, p. 204; Prescott, S. C.: Science, N. S., 1902, 15, p. 363; Prescott, S. C.: Medicine, 1903, 11, p. 20; Prescott, S. C.; Biological Studies by the Pupils of William Thompson Sedgwick, 1906, p. 208. Prescott and Winslow: Elements of Water Bacteriology, New York, 1913.

[2] Jour. Med. Research, 1908, 19, p. 175.

[3] Jour. Infect. Dis., 1915, 17, p. 160. Jour. Bacteriol., 1917a, 2, p. 1.

[4] Ibid., 18, p. 358.

[5] Jour. Bacteriol., 1916, 1, p. 81.

which correlated the above characters with the Voges-Proskauer reaction. Thus the colon family may at present be divided into two general groups depending on these three closely correlated characteristics, namely:

(1) B. coli type giving a low gas ratio, positive methyl-red and negative V-P tests.

(2) B. aerogenes type giving a high gas ratio, negative methyl-red and positive V-P tests.

It must, however, be remembered that these two divisions include members whose fermentative powers toward different sugars, alcohols, etc., may be widely unrelated. For instance, we find organisms giving a positive Voges-Proskauer reaction (characteristic of the B. aerogenes group) distributed throughout three of the four primary fermentative groups of MacConkey's classification. The same thing is seen in Kligler's[6] work on the classification of this group. The investigations conducted prior to the introduction of the methyl-red test can therefore be correlated with present information only by means of the Voges-Proskauer reaction.

From a practical sanitary standpoint the important question is whether the normal habitats of the B. coli and B. aerogenes types and their relative distribution in nature are so different as to warrant the placing on their presence in water and food products of a distinct and different interpretation.

Booker,[7] Hammerl,[8] Hellström,[9] and many others report both B. coli and B. lactis-aerogenes (one of the commonest representatives of the B. aerogenes group) as present in human stools. MacConkey[1] was unable to isolate by the enrichment method any of the B. lactis-aerogenes group from a normal specimen of feces—the 36 cultures isolated were all V-P negative. He examined other specimens with the special purpose of isolating B. lactis-aerogenes, but in these he did not use the V-P test, presumably depending on sugar fermentations for identification. He found only 4 out of 241 strains from human feces to be definitely of the fermentative B. lactis-aerogenes type. He found no B. cloacae present. He was also struck by the absence of B. lactis-aerogenes in a series of 99 cultures isolated from horse, cow, and calf feces.

Ferreira, Horta and Paredes[10] studied 117 strains of lactose fermenting bacilli from human feces. The 'Proskauer reaction' (apparently the Voges-Proskauer reaction, though it is not quite clearly stated) was positive in glucose broth only 8 times out of the 117 cultures. This would indicate that the B. aerogenes group was represented in their collection to the extent of 6.8%. MacConkey

[6] Jour. Infect. Dis., 1914, 15, p. 187.

[7] Centralbl. f. Bakteriol., 1891, 10, p. 284. Bowles, J. T. B.: Am. Jour. Pub. Health, 1916, 6, p. 1173.

[8] Centralbl. f. Bakteriol., 1897, 1, Ref. 22, p. 706.

[9] Ibid., 30, p. 309.

(1909) in a further study found 11 cultures, or 6.2% of 178 strains isolated from human feces to give a positive Voges-Proskauer reaction. In horse feces he found 8 cultures, or 11.9% of 67 strains to be Voges-Proskauer positive. In 87 strains from calf, goat, pig, and goose feces he records no Voges-Proskauer positive cultures. In addition, he reports 68, or 56.2% Voges-Proskauer positive strains out of a total of 121 bacilli isolated from corn ear, malt, beans, oats, cheese, rain water, roof washings, pond water, and soils, the latter representing different degrees of possible contamination. Houston[11] gives a series of data on cultures isolated from waters. Among the tests are included lactose fermentation and the V-P reaction. From these we have calculated the percntage of lactose fermenters in his series which gave a positive V-P reaction, and find that about 13% of the strains from raw water, 5% of those from stored water, and 3% of those from filtered water were of this type. Clemesha[12] made an extended study of the colilike organisms in human and bovine feces. Nine series were run with the human feces through a period of 3 years; 104 samples of feces were examined, and the results are based on 1,207 cultures isolated. There were 10 series of cow feces through the same period, comprising 86 samples and 1,029 cultures isolated therefrom. The average percentages of the Voges-Proskauer positive organisms recorded as compiled from his results were 6.06% for the human and 10.66% for the bovine strains.

The next series of extended researches is that of Rogers and his co-workers. In 1914 Rogers, Clark and Evans[13] found but one out of 150 strains isolated from bovine feces to be of the high ratio, B. aerogenes, type. Later,[14] the same authors, in a study of 166 organisms isolated from grains found 151 to be of the high ratio, B. aerogenes type. They note that the types found on grains are characterized by pigment formation on agar. Rogers, Clark and Lubs[15] in a study of the colon bacteria in human feces found 107 out of 113 to be of the low ratio, B. coli, type, the remaining 6 being of the type corresponding to the predominating group in the grain cultures reported above. They conclude, however, that "while this (grain) type occurred in relatively small numbers (in feces), the actual number may amount to several hundred thousand in each gram of material. It is possible that the more frequent occurrence outside of the animal body of the high ratio type may be because it is more resistant to unfavorable conditions and consequently survives after the low ratio type disappears." The same authors in a more recent paper (unpublished manuscript, 1917) include 63 more cultures of which 40 were high ratio cultures. Out of the total of 176 strains 46, or 26%, were therefore of the B. aerogenes type. Rogers[16] examined the types of colon bacilli occurring in surface waters, and found out of 137 strains isolated, about one-third of the low ratio, B. coli, type. This form was found occasionally in springs in which there was no evident source of contamination, but was especially abundant in streams polluted with sewage. He states that the significance of the presence of this type in water cannot be determined.

Levine[17] in a study of 187 cultures from sewage and feces found that only 28 gave a positive V-P reaction. He states that the colilike bacteria which give this reaction are characteristically of nonfecal origin, that they resemble B.

[10] Arc. D. R. Inst. Bact. Camara Pestana, 1908. Tome II, Fasc. II, p. 153.
[11] Seventh Research Report to Metr. Water Board, London, 1911.
[12] The Bacteriology of Surface Waters in the Tropics, 1912.
[13] Jour. Infect. Dis., 1914, 15, p. 100.
[14] Rogers, Clark and Evans: Jour. Infect. Dis., 1915, 17, p. 137.
[15] Jour. Bacteriol., 1916, 1, p. 82. Unpublished manuscript, 1917.
[16] Ibid., p. 82.
[17] Ibid., p. 87.

aerogenes (Escherich) and probably represent soil forms. In another paper[18] this author states: "The Voges-Proskauer reaction is of considerable sanitary significance. It differentiates between fecal and nonfecal colilike organisms and may be an index of soil washings." In still another communication[19] he reports that the methyl-red negative, V-P positive organisms were not uncommon in sewage, but rarely present in the feces of man, horse, sheep, pig, and cow. Johnson[20] in an examination of 363 colilike organisms from the soil (of Iowa) found the prevailing organisms to be of the 'nonfecal,' B. aerogenes, type. He has failed, however, to report a perfect correlation between the negative methyl-red test and positive V-P reaction as others have done. Of the 261 methyl-red negative organisms in his collection, only 84% gave a positive V-P reaction. Hulton[25] (1916) studied 45 coliform organisms of which only 12 were isolated from feces, and did not find any of the B. aerogenes type among the latter. The author concludes from this observation that the methyl-red negative, V-P positive reactions are of importance in determining colon organisms of fecal origin—a rather broad conclusion from an apparently limited study. A rather suggestive paper is one by Greenfield[21] on the soil and fecal types of the colon-aerogenes group in waters of Kansas. This author states in the account of her methods of isolation that all cultures that failed to liquefy gelatin were discarded. This is undoubtedly a misprint, and we assume it was meant that all gelatin liquefiers were discarded. This fact should be kept in mind for these organisms, representing the B. cloacae group, if included, would have increased the percentage of methyl-red negative, V-P positive organisms in the final results as reported.

The very interesting results obtained are as follows:

24% of 116 strains from raw waters were methyl-red negative
30% of 131 strains from treated waters were methyl-red negative
35% of 158 strains from ground waters were methyl-red negative

That is to say, the greater proportion of cultures studied were of the so-called true fecal type. On the other hand, 68% of 19 strains from natural ice were of the B. aerogenes group, which would seem to suggest that it may be more resistant to freezing temperature than the B. coli type.

In Table 1 are summarized the most significant findings in the work reported by various observers on feces and sewage, water, soil, grains, etc.

A brief survey of Table 1 indicates that the percentage of V-P +, M.R.— organisms in feces is not constant, and is, in general, small. The evidence also seems to show that on grains and cereals the B. aerogenes type is the predominating one. As to the prevalence of these types in soils, the facts are conflicting. Houston was unable to find any of the colon family in his studies in England. Clemesha was

[18] Levine, M.: Jour. Bacteriol., 1916c, 1, p. 153.
[19] Levine, M.: Jour. Infect. Dis., 1916a, 18, p. 358.
[20] Jour. Bacteriol., 1916, 1, p. 96.
[21] Jour. Infect. Dis., 1916, 19, p. 647.

able to isolate B. cloacae easily, while Burton and Rettger[22] found the predominating gas formers in soils to be apparently B. cloacae, not B. aerogenes. Except for the few researches recorded in the accompanying table, evidence in regard to the prevalence of these two types in waters of different sanitary quality is almost wholly lacking. Recently, the feeling has been growing that B. coli represents a true fecal type characteristic of undesirable pollution in waters, while the B. aerogenes type in water is derived from the soil, and represents a nonfecal source. It is perhaps fair to state that Rogers has carefully refrained from making such a broad statement, in spite of his very suggestive work, though Levine, Hulton and others apparently accept it as proven.

TABLE 1

SUMMARY OF RESULTS OF VARIOUS INVESTIGATORS IN REGARD TO THE PREVALENCE OF B. AEROGENES IN VARIOUS MATERIALS

Authors	Source	No. Strains	Per Cent. V-P+ or M. R.-
	Feces		
Ferreira, Horta, and Paredes (1908)...	Human	117	6.8
MacConkey (1905)	Human	36	0
MacConkey (1909)	Human	178	6.2
MacConkey (1909)	Horse	67	11.9
	Other animals	87	0
Clemesha (1912)	Human	1,207	6.1
Clemesha (1912)	Cow	1,029	10.7
Rogers, Clark, and Evans (1914)	Cow	150	0.7
Rogers, Clark, and Lubs (1916)	Human	113	5.6
Rogers, Clark, and Lubs (unpublished)	Human	177	26.0
Levine (1916)	Sewage	...	23.0
	Feces	187	0
MacConkey (1909)	Cereals, waters, etc.	121	56.2
Houston, A. C. (1911)	Raw water	248	12.9
	Stored water	133	5.3
	Stored and filtered water	156	3.2
Rogers, Clark, and Evans (1915)	Grains	166	91.0
Rogers (1916)	Water	137	33.3
Greenfield (1916)	Water	405	30.0
Johnson (1916)	Soil	363	72.0*

* The percentage is figured on the results of the methyl-red test, not the V-P test.

This is the present state of the subject. The object of the present investigation was to add to the scanty evidence in regard to the relative prevalence of the B. coli and B. aerogenes types in waters of known sanitary quality in order to throw light on the significance to be attached to their presence. In other words, our object was to see if there exists a correlation between the character of the source and type of the coliform organisms isolated from waters in the neighborhood of New Haven, Conn.

[22] Jour. Infect. Dis., 1917, 21, p. 162.

COLLECTION OF SAMPLES

The samples of water collected in this work were classified under three heads: (1) samples of water that were known to be polluted; (2) samples that were believed to be unpolluted, and (3) samples of stored raw waters used as municipal supplies. The decision as to whether a water is free from pollution or not is somewhat difficult, but our criteria were very strictly drawn to reduce the uncertainty to a minimum. The samples of unpolluted waters were taken from areas that were far removed from human habitation, and apparently from contamination from human or animal sources. One series for example was secured from Mt. Carmel, several hundred feet above the surrounding country, the samples being taken from rocky pools, or as the water flowed from under some overhanging sandy or rocky banks. In several cases, the drip was collected from precipitous earth banks covered with luxurious green growth. The possibility of human or animal contamination in such instances was very far removed. We might also record that these samples were taken during the autumn of 1916 when there was no very heavy rainfall, and therefore little chance of pollution being introduced by surface wash. The chance for contamination by air and dust is of course not excluded except in those samples taken directly from springs. Another set of samples was taken from a water-bearing area near Mt. Sandford, about 10 miles northwest of New Haven. This is situated several miles from any well-traveled roads, on a thickly wooded slope and well covered with underbrush. The probability of chance pollution here was highly remote. Most of these water samples were taken from springs as they bubbled out of the ground, thus offering no chance for even casual contamination by dust. This series constituted the greater proportion of the unpolluted samples. Others were taken throughout the country, from grassy woodland spring-pools, and at the headwaters of streams. In all cases a careful survey was made of the whole ground before a sample was accepted as fulfilling our requirements. To illustrate: at one place, we came across a piece of woods through which a crystal clear brook ran. It was far from any dwellings, etc. and seemed in many ways a desirable source. Several samples were taken at suitable points as we pursued our customary survey to the headwaters. We were, however, surprised to find a small pond dammed off near the origin of this stream, and on top of the steep banks, two dwellings and a stable, whose drainage provided part of the stream flow. We also learned that the pond was used as a swimming pool. It is needless to say that our samples from this stream were discarded.

The samples of polluted water were easier to obtain. These comprised pools in barnyards, diluted sewage carried in the West and Quinnipiac Rivers, and ponds that very visibly received drainage from dwellings, barnyards, and privies along the banks. It should of course be kept in mind that the rivers carrying sewage also undoubtedly received drainage from unpolluted sources as well. We also included three cultures isolated from oysters taken in a polluted harbor. About two-thirds of the samples from stored lake and river waters were secured from the New Haven city water supply. Seven cultures were isolated during a routine examination of the city supply. The other samples were collected in the city at a short distance from the Maltby Lakes supply. This water was untreated, and came from a series of three large storage lakes of high sanitary quality. We encountered much difficulty in getting coliform organisms from this source in 100 c c samples and had to resort to concentration methods on larger volumes of water. This is rather surprising, as these samples were taken during the early spring when one would expect to find

sewage organisms most numerous in waters. The following apparatus was devised and proved quite effective as well as practical.

The apparatus used for concentrating the bacteria from this stored water was made up of galvanized iron fittings usually to be found in plumbers' shops. It consists of a 1¾ inch galvanized iron pipe of suitable length, threaded at both ends; a cap to fit the lower end and a reducer the upper. The cap has a ¼ inch hole bored in the center. The reducer tapers so as to fit a 1 inch threaded faucet (for connection of garden hose); and when not in use a 1 inch plug is used to close the tapered end. The upper end is closed with a perforated rubber stopper; and a porous clay filter of suitable size is inserted so that its outlet tube passes through the stopper and the hole in the cap when the cap is screwed on tightly. The outfit may thus be easily handled, autoclaved, and transported wherever necessary.

When it is desired to use the apparatus, the faucet from which the sample is to be secured is rendered sterile by flaming. The plug at the tapered end is removed aseptically, and the apparatus attached to the faucet. The water may now be turned on at full pressure for filtration. Because of its compactness and strength, the apparatus will effectively withstand a high pressure and in consequence give a high rate of filtration. We were able with a 6 inch Berkefeld candle to obtain a filtration rate of 1 liter in 5 minutes. A much higher rate no doubt might be obtained without danger. When the required volume of water has been filtered, the apparatus is unscrewed from the faucet, and the plug tightly replaced. The outfit may now be removed to the laboratory for examination. The water in the annular space (about 50 c c) is examined. The chamber is rinsed with sterile water, and the rinsings examined, and finally, the candle is removed aseptically and placed in a cylinder of lactose broth for enrichment and the resulting growth examined. As a rule we filtered 5 liters of water for a sample.

Fifteen cultures representing stored raw waters were obtained from the laboratory of the New Haven Water Company as isolated in routine examination. Eleven of the same type were obtained from the Connecticut State Board of Health Laboratory. These represent the only cultures that were not directly isolated by ourselves. They were isolated, however, by methods that were very closely, if not exactly, like our own.

METHOD OF ISOLATION

The water to be examined was in all cases inoculated into various dilutions of standard lactose broth and incubated at 37 C. for 24-48 hours. The highest dilutions showing gas were streaked on azolitmin-lactose-agar plates which were then incubated at 37 C. These plates were examined from 12-24 hours later and acid colonies picked off and studied for further characteristics of the colon group. For reasons that have been clearly brought out by Prescott and Winslow (1913) the following characters were considered sufficient to permit an organism to enter our collection as a member of the colon-aerogenes group. The organism had to be a short, thick rod, non-spore-forming, aerobic and gram-negative. Further, it had to produce acid and gas in a lactose fermentation medium.

In our work we have assumed that the preliminary enrichment of the samples would not alter to any great extent the relative proportions of the two main types of coliform organisms should they both be present. MacConkey (1905) thinks that such a preliminary incubation in a liquid medium might alter the relations somewhat by favoring the growth of the B. lactis-aerogenes group. This point might be kept in mind when considering our own results on the

occurrence of the aerogenes types in these samples. The cultures were kept on standard nutrient agar slants at room temperature (about 18 C.) and transferred to fresh slants once a month. Tests made at the beginning and end of this research showed no apparent change in characters due to storage.

DISTRIBUTION OF COLIFORM ORGANISMS IN WATERS OF DIFFERENT TYPES

By these methods, we succeeded in isolating 255 strains distributed according to source as follows:

94 cultures from polluted waters
80 cultures from unpolluted waters
81 cultures from stored raw waters

It should be remembered that not all samples yielded cultures. This was especially the case with the samples from the unpolluted sources, about 100 samples proving negative. An important point is brought out by considering the dilutions from which these cultures were isolated in relation to the character of the source as given in Table 2.

TABLE 2

FREQUENCY OF ISOLATION FROM DIFFERENT DILUTIONS

	100 C C	10 C C	1 C C	1/10 C C	1/100 C C	1/1,000 C C	1/10,000 C C
Polluted, number.....	2	18	36	25	7	5	1
per cent.....	2	19	38	27	8	5	1
Unpolluted, number....	51	24	4	1			
per cent....	64	30	5	1			

The cultures from the stored waters are not included because of the different method of procedure in securing them. The greater number of cultures from unpolluted sources were isolated from 100 c c samples, and 94% of them came from 100 c c or 10 c c samples. On the other hand, in the polluted samples, most of the cultures were isolated from 1 c c and 0.1 c c, the incidence extending down to 0.0001 c c. These data testify both to the value of the test for the whole group of coliform organisms as a measure of pollution and to the validity of our judgment as to the actual sanitary quality of the waters examined.

CULTURAL AND BIOCHEMICAL CHARACTERS OF THE ORGANISMS STUDIED

Morphology and Motility.—Young 18-24 hour broth cultures were examined in hanging drop preparations. The majority of the cultures showed short thick rods with rounded ends. A few appeared rather slender. Some were exceedingly short and coccoid in shape. Motility was also observed in the hanging drop. About two-thirds of the collection were positively motile, and about equally distributed through the three classes of samples.

Gram Stain.—This was made on smears of the young cultures just mentioned. The stain was applied for 1 minute and the smear decolorized for 5 minutes. All the cultures in this series were gram-negative, although a few seemed to retain slight traces of the stain.

Indol Production.—The formation of indol in standard extract broth (Difco peptone) was determined after incubation at 37 C. for 4 days. The Ehrlich

test (para-dimethylamidobenzaldehyde + HCl) was used as it is said to be more reliable than the Salkowski test ($H_2SO_4 + KNO_2$). Sixty-seven, or 71% of the strains from polluted waters formed indol against 41, or 51% of those from unpolluted waters, and 57, or 62%, of those from stored waters. We do not know the exact significance of the indol test from the sanitary viewpoint. Houston places some reliance on it in determining his 'typical' coli organisms. Our results would indicate that the fewest indol producers are found in unpolluted waters, while the polluted sources give the most, the stored waters coming almost exactly in the middle between these two values.

Gelatin Liquefaction.—This was determined by spreading a loopful of 24-hour broth culture on the surface of nutrient gelatin and incubating at 20 C. for 30 days. Another series incubated for 10 days at 37 C. and then chilled in the ice-chest gave identical results. Of the strains from polluted waters 4, or 4%, liquefied gelatin, of those from unpolluted waters 2, or 2%, and of those from stored waters 4, or 5%. The gelatin liquefiers include the B. cloacae group, which is declared by Clemesha to be present in feces in varying numbers, but to be the most resistant type of coliform bacteria and capable of remaining for a long time in water and soils. This type occurred in very small numbers in our series and we are therefore unable to judge as to its probable significance.

Fermentation with Gas Production.—This was observed in standard lactose and glucose broths in Durham fermentation tubes incubated at 37 C. for 48 hours. All the cultures attacked these sugars with the formation of gas. Three of them, however, did so rather slowly.

Limiting Hydrogen Ion Concentration.—This was determined in the 0.5% Witte peptone, dipotassium phosphate-glucose medium of Clark and Lubs. (Incubation being at 37 C. for 4 days.) The final P_H was determined for each culture by the colorimetric procedure described by Clark and Lubs[3] (1917a). The standard phthalate-NaOH solutions were compared with Walpole's acetic acid-sodium acetate mixtures and checked up perfectly. The indicators used were methyl-red and brom-thymol blue. The methyl-red positive cultures gave a final P_H ranging from 4.6-5.4; the methyl-red negative cultures gave a final P_H from 6.4-7.2 in the methyl-red medium previously described. We encountered serious difficulties, however, when Difco peptone was inadvertently submitted for Witte's. A number of the cultures gave neutral tints with the methyl-red, while others of the B. aerogenes group produced a high limiting acidity. We have reason to believe that the degree of purity of the sugar may be another contributory factor in such peculiar behavior. Through the courtesy of Dr. Clark, who kindly furnished the crystalline materials, we were also enabled to run our cultures through the new synthetic medium described by Clark and Lubs.[23] The results were exactly the same as those obtained in the Witte's peptone medium, the only variation being that the ranges of the 2 types (methyl-red + and —) were placed closer together in the P_H scale. Thus, the methyl-red positives ran from 4.8-5.6 while the methyl-red negatives ran from 5.8-6.6. Several of the cultures gave neutral tints that were not easy to read, but the differentiation between the 2 types was quite clear. Two or three of the cultures did not grow as readily in this medium as in the other. In the main, however, we have found it to be exceedingly satisfactory for this work. In addition to the simplicity of preparation, there was an entire absence of inherent color after sterilization which is a desirable thing in colorimetric work. Classified according to the results obtained with the methyl-

[23] Bact. Abstr., 1917b, 1, p. 29.

red test, 72, or 77%, of the strains from polluted waters, 61, or 76%, of those from unpolluted waters, and 69, or 85%, of those from stored waters were positive, or of the B. coli type.

These results do not seem to indicate any close correlation between the source and type of coliform organism found therein. There seems to be an almost equal distribution of both types in polluted and unpolluted waters, and a somewhat greater proportion of the methyl-red positives in the stored waters. This is in fair agreement with the results obtained by Greenfield on the waters in Kansas. Our results also correspond in a way with the data we have quoted from Houston.[11]

The Voges-Proskauer Reaction.—This test was carried out in glucose broth which was incubated for 4 days at 37 C. by the addition of 10% NaOH, observing the development of the characteristic eosin-like coloration that gradually appeared. The tubes were not discarded as negative until a period of 24 hours had elapsed after addition of the reagent without the appearance of the coloration. Some of the tests were brilliant, but there were a number that were not so marked and left much to be desired. We must admit that if we had not been on the lookout for it in some of the B. aerogenes cultures, it would have gone by unnoticed. We have one culture (isolated from a polluted oyster) that persists in giving a positive V-P reaction, though it is a methyl-red positive organism. We have found, however, that there is in general an almost perfect correlation between the negative methyl-red test and a positive Voges-Proskauer reaction. The organisms giving a positive Voges-Proskauer made up 24% of the samples from polluted waters, 24% of those from unpolluted waters, and 15% of those from stored waters.

Rogers and his co-workers point out that there appears to be some difference between the typical B. aerogenes cultures isolated from grains and those from feces. They found that a large number of the grain cultures produced a noticeable pigment on nutrient agar and that only a small percentage of them attacked adonite. On the other hand, the B. aerogenes strains from feces produced only slight pigment and all vigorously attacked adonite. It therefore seemed desirable to test our B. aerogenes collection for these characters to see how far they agreed with Rogers' strains in relation to their source.

Pigment Formation.—The cultures were grown on agar for 15 days at 20 C. As much as possible of the growth was then removed with a spatula to white drawing paper and compared at once with the plates in Ridgway's "Color Standards and Nomenclature." With one exception, all the cultures produced no marked pigment. Most of them matched the "Cartridge Buff" or "Ivory Yellow" in the above plates. The exception was a culture isolated from a polluted brook running through a farmyard. It was also a strong gelatin liquefier. This culture gave a pigment one shade darker than "Honey Yellow" or 19″YO-Yi on Plate XXX.

Adonit Fermentation.—This was determined in a medium made according to the following formula: Water, 100 gm.; peptone (Difco), 1 gm.; beef extract, 0.4 gm.; dibasic potassium phosphate, 0.5 gm.; adonit, 1 gm. The cultures were incubated at 37 C for 6 days and then titrated. They were all methyl-red negative on addition of this indicator. We did not find any acid production where gas production was absent in this series. Of the B. aerogenes strains from polluted waters 13, or 59%, fermented adonit, of those from unpolluted waters, 17, or 90%, and of those from stored waters, 7, or 58%.

(The methyl-red positives were not tested for adonit fermentation.)

A greater proportion of the B. aerogenes from the unpolluted sources attacked adonit than did those from the polluted waters, although the numbers are too small to be significant. If the adonit fermentation may be taken as an index of B. aerogenes of fecal type, our results as far as they go would seem to show that the fecal type is prevalent in unpolluted sources. This is also borne out by the statement of Rogers[24] that there is a very much closer agreement in group characteristics between the B. aerogenes cultures from water and from feces than between the water and grain cultures.

CORRELATION OF BIO-CHEMICAL CHARACTERS IN THE SERIES OF CULTURES STUDIED

In the course of this study we have examined 255 strains of coliform organisms; of which 202 were methyl-red positive and 53 methyl-red negative. The correlation of other biochemical characters with the methyl-red reaction is indicated in Table 3.

TABLE 3

CORRELATION OF OTHER BIO-CHEMICAL CHARACTERS WITH THE METHYL-RED REACTION

Methyl-Red Reaction	Percentage of Cultures in Each Class			
	Voges-Proskauer Positive	Indol Formed	Adonit Fermented	Gelatin Liquefied
+	0.5	71.8		1.5
−	100.0	37.7	70.0	13.2

The Voges-Proskauer reaction is almost perfectly correlated with a negative methyl-red reaction, while indol formation is more common with the B. coli strains and gelatin liquefaction with the B. aerogenes strains, facts in close accord with the results of earlier workers.[25]

DISTRIBUTION OF B. COLI AND B. AEROGENES TYPES IN WATER OF DIFFERENT SANITARY QUALITY

In general our study, as indicated by Table 4, did not reveal any marked difference between the characters of coliform organisms from different sources.

Our study of a limited number of cultures isolated from polluted, nonpolluted, and stored waters does not therefore seem to show any connection between the type of organism and corresponding source. The final proof or disproof of any such connection must come from extended study in as many different parts of the country as possible. Hence, this study must not in any way be considered as definitive. We can only say that, under the conditions of our work at New Haven, no correlation between source and type of organism was discovered.

[24] Bact. Abstr., 1917, 1, p. 56.
[25] Jour. Infect. Dis., 1916, 19, p. 606.

An objection might be raised against our method of taking one single organism as representative of a sample, which is valid where the polluted samples are concerned. The cultures isolated from the nonpolluted sources are most important, however, and since no coliform organisms were obtainable in lower dilutions than 100 c.c. in a majority of these samples, we can fairly presume that an organism isolated from that volume of water, and not found in smaller volumes, must on the average come fairly close to being a representative of the prevailing type. The fact that of these organisms from unpolluted sources three quarters were of the B. coli type is therefore of significance. It would be highly desirable, however, to know the actual ratio of B. coli to B. aerogenes types in a given sample. To obtain data of this sort would be a time-consuming process, and we have not attempted it in this study.

TABLE 4

PRECENTAGE OF CULTURES FROM WATERS OF DIFFERENT QUALITIES EXHIBITING VARIOUS CHARACTERISTICS

Type of Water	Methyl-red Negative	Voges-Proskauer Positive	Indol Not Formed	Adonit* Not Fermented	Gelatin Liquefied
Polluted	23	24	29	41	5
Unpolluted	24	24	49	10	3
Stored	15	15	38	42	5

* This test applied only to 53 methyl-red negative strains.

That the B. coli type is the predominant one in feces and the B. aerogenes the predominant one on grains there seems no reason to doubt from the results of Rogers and his co-workers. What our data indicate, however, is that the grain or soil forms do not as a matter of fact gain access to the natural waters about New Haven in such numbers as to increase the relative prevalence of this type as compared with the conditions which obtain in more polluted waters.

We may of course rest safely on the general conclusion that the quantitative test for gas forming aerobes of all sorts is an admirable guide to the sanitary quality of water. Our own data tend to confirm this conclusion if any confirmation were necessary. If similar results to ours are obtained in other regions it would seem that the further differentiation between the B. coli and B. aerogenes may not be of special importance in determining the sanitary quality of water. If, however, in a given sample a large proportion of the B. aerogenes types were actually found, the results might perhaps be interpreted as signifying that the gas-forming organisms present were presumably not of recent fecal origin.